



## **The histamine H1 receptor is not involved in local control of mammary blood flow in dairy cows**

Madsen, Torben Gosvig; Trout, D.R.; Cieslar, S.R.L.; Purdie, N.G.; Nielsen, Mette Benedicte Olaf; Cant, J.P.

*Published in:*  
Journal of Dairy Science

*DOI:*  
[10.3168/jds.2007-0845](https://doi.org/10.3168/jds.2007-0845)

*Publication date:*  
2008

*Document version*  
Publisher's PDF, also known as Version of record

*Citation for published version (APA):*

Madsen, T. G., Trout, D. R., Cieslar, S. R. L., Purdie, N. G., Nielsen, M. B. O., & Cant, J. P. (2008). The histamine H<sub>1</sub> receptor is not involved in local control of mammary blood flow in dairy cows. *Journal of Dairy Science*, 91, 2461-2468. <https://doi.org/10.3168/jds.2007-0845>

## The Histamine H<sub>1</sub> Receptor Is Not Involved in Local Control of Mammary Blood Flow in Dairy Cows

T. G. Madsen,\* D. R. Trout,† S. R. L. Cieslar,‡ N. G. Purdie,‡§ M. O. Nielsen,\* and J. P. Cant‡<sup>1</sup>

\*Department of Basic Animal and Veterinary Sciences, The Faculty of Life Sciences, Copenhagen University,

DK-1870 Frederiksberg C, Denmark

†Department of Clinical Studies, and

‡Department of Animal and Poultry Science, University of Guelph, Ontario N1G 2W1, Canada

§Schools of Animal Studies and Veterinary Science, University of Queensland, St. Lucia 4072, Brisbane, Australia

### ABSTRACT

Low concentrations of the essential amino acid histidine in circulation have been shown to increase mammary blood flow and it has been suggested that this effect is mediated by histamine. The hypotheses tested in this experiment were that interstitial histamine concentrations in the mammary gland are related to arterial His concentrations and that mammary blood flow is reduced by extracellular histamine via H<sub>1</sub> receptors. The hypotheses were tested by infusing saline or chlorpheniramine, a blocker of the H<sub>1</sub> histamine receptor, into the arterial supply of the mammary glands of lactating cows infused with 44 g/h of amino acid mixtures with or without His for 10 h. Infusates were administered in a 2 × 2 factorial arrangement within a 4 × 4 Latin square to 4 multiparous Holstein cows in mid lactation. Exclusion of His from the infusate decreased protein content in milk from the infused udder half from 3.98 to 3.77%, and increased arterial  $\alpha$ -amino-nitrogen concentration from 3.2 to 3.4 mM. Neither the decreased arterial His concentration nor the H<sub>1</sub> blocker affected plasma flow to the infused udder half. We conclude that histamine is not involved in the regulation of mammary blood flow. The H<sub>1</sub> blocker decreased milk production in the infused udder half from 4.6 to 3.5 kg without affecting protein, fat, and lactose percentages, suggesting an inhibition of milk ejection. Cows on chlorpheniramine ate less feed during the infusion than saline-infused cows, which resulted in lower arterial concentrations and mammary uptakes of acetate. The efficiency of plasma triacylglycerol uptake across the mammary glands was decreased by chlorpheniramine but net uptake of long-chain fatty acids was not affected. The mechanism by which an amino acid deficiency influences mammary blood flow does not involve

histamine signaling through the H<sub>1</sub> receptor and remains unidentified.

**Key words:** histidine, histamine, mammary plasma flow, dairy cow

### INTRODUCTION

To sustain milk synthesis the mammary gland depends on an adequate vascular supply of nutrients, where mammary blood flow is thought to play an important role (Davis and Collier, 1985). In general, mammary blood flow correlates closely with milk production in ruminants (Linzell, 1974; Nielsen et al., 1990), which indicates that mammary blood flow, like flow to other tissues, is related to the metabolic activity of the mammary gland (Prosser et al., 1996). By extension, if circulating concentrations of milk precursors affect metabolic activity of the mammary gland, then they should also affect its rate of blood flow. When circulating concentrations of glucose were increased from 3.6 to 6.4 mM by close arterial infusion for 10 h, mammary plasma flow decreased by 16% (Cant et al., 2002). Similarly, when circulating concentrations of the essential amino acid His were reduced from 73 to 8  $\mu$ M by abomasal infusion of a His-lacking amino acid mix for 6 d, mammary plasma flow increased by 33% (Bequette et al., 2000).

Blood flow rate depends on vascular resistance to the arteriovenous pressure gradient. Locally produced compounds such as adenosine, CO<sub>2</sub>, prostaglandins, and NO have been shown to relax the resistance to blood flow in the mammary glands (Linzell, 1974; Nielsen et al., 1995; Lacasse et al., 1996). It has been proposed that the nutrient effect on mammary blood flow is related to the interstitial release of such vasoactive agents as a consequence of over- or underproduction of ATP relative to its utilization (Cant and McBride, 1995; Cant et al., 2003). According to this ATP balance hypothesis, Cant et al. (2003) suggested that decreased plasma concentrations of the energy metabolites acetate and  $\beta$ -hydroxybutyrate, characteristic of an amino acid defi-

Received November 8, 2007.

Accepted February 22, 2008.

<sup>1</sup>Corresponding author: jcant@uoguelph.ca

ciency in lactating cows (Weekes et al., 2006), could account for the observed hyperemia in His-deficient goats (Bequette et al., 2000). However, energy metabolites and mammary blood flow have not been measured simultaneously in the same His-deficient animals, so the explanation remains tenuous at best.

An alternative explanation of the low His-induced hyperemia is due to local production of histamine, as histamine has been shown to have vasoactive effects in several tissues (Buckley et al., 1988; Parsons et al., 1992). Histamine can be both vasoconstrictive and vasodilatory. Vasodilation appears to be due to release of relaxation factors from the vascular endothelium upon activation of either  $H_1$  or  $H_2$  receptor (Jin et al., 2006; Stähli et al., 2006). Vasoconstriction, on the other hand, is mediated in the absence of endothelium by  $H_1$  receptors (Sato and Inui, 1984; Jin et al., 2006; Stähli et al., 2006). At low concentrations, histamine dilated mammary arteries from humans in vitro, but constricted at greater concentrations (Stähli et al., 2006). At all concentrations, histamine exerted contractile effects on mammary arteries from nonlactating goats (Jakobsen et al., 1994). The enzyme for synthesis of histamine from His, histamine decarboxylase, has been detected in mast cells and epithelial cells of the bovine and murine mammary gland (Maslinski et al., 1993). If histamine release into the mammary interstitium is directly related to arterial His concentration and histamine is constrictive to mammary blood flow, then a temporary His deficiency would be expected to increase mammary blood flow.

The hypotheses tested in this experiment were that interstitial histamine is related to arterial His concentration and that mammary blood flow is reduced by extracellular histamine via  $H_1$  receptors. The hypotheses were tested by infusing chlorpheniramine (CPA), a blocker of the  $H_1$  histamine receptor, into the arterial supply of the mammary glands of lactating cows given amino acid mixtures with or without His for 10 h. If His is constrictive to mammary blood flow via  $H_1$ -receptors, then CPA should increase flow.

## MATERIALS AND METHODS

All animal procedures and holding facilities were approved by the Animal Care Committee at the University of Guelph. Four second-lactation Holstein cows at  $223 \pm 20$  DIM and averaging  $606 \pm 11$  kg of BW and  $30 \pm 1$  kg/d milk were assigned to the present experiment. The cows were fitted with polyurethane catheters in both subcutaneous mammary abdominal veins and with polyethylene catheters in both external iliac arteries via the saphenous arteries. The external iliac posi-

**Table 1.** Chemical composition of feedstuffs

| Item   | Concentrate | Hay  | TMR  |
|--|-------------|------|------|
| Composition of TMR, % of as fed                | 44          | 56   |      |
| DM, % of as fed                                | 87.2        | 90.0 | 88.8 |
| CP, % of DM                                    | 11.2        | 16.9 | 14.4 |
| Buffer solubility, % of CP                     | 24.8        | 47.6 | 40.0 |
| NDF insolubility, % of CP                      | 5.8         | 16.4 | 12.9 |
| ADF insolubility, % of CP                      | 1.8         | 8.5  | 6.3  |
| NDF, % of DM                                   | 10.4        | 53.3 | 34.8 |
| ADF, % of DM                                   | 3.3         | 43.1 | 25.9 |
| Cellulose, % of DM                             | 3.3         | 36.1 | 21.9 |
| Lignin, % of DM                                | 0.0         | 7.0  | 4.0  |
| Fat, % of DM                                   | 8.1         | 0.7  | 3.9  |
| Ash, % of DM                                   | 4.4         | 8.3  | 6.6  |
| Ca, % of DM                                    | 0.56        | 0.80 | 0.71 |
| P, % of DM                                     | 0.47        | 0.30 | 0.38 |
| Mg, % of DM                                    | 0.17        | 0.20 | 0.19 |
| Calculated $NE_L$ , Mcal/kg of DM <sup>1</sup> | 2.06        | 1.20 | 1.57 |

<sup>1</sup>Calculated according to NRC (2001).

tion was confirmed by injection of Evans blue into the arterial catheter (Cant et al., 2001).

Cows were housed in tie stalls bedded with wood shavings. They were milked and fed a TMR low in protein at 0800 and 1800 h daily. The ration was composed of 44% concentrate (85% corn, 5% soybean meal, 5% tallow, and 5% mineral mix on a DM basis) and 56% chopped, mixed hay (Table 1). Concentrate and hay were sampled at each feeding and composited for analysis at a commercial laboratory. Before every feeding, orts were removed and weights recorded. Milking was performed with a modified bucket milker to collect milk from each udder half separately.

The experiment was conducted in 2 blocks, with 2 cows in each block, because the facilities only allowed for sampling from 2 cows at a time. Each cow was randomly assigned to 4 treatments on 4 consecutive days in a balanced  $4 \times 4$  Latin square design. The 4 treatments were a  $2 \times 2$  factorial arrangement of arterial infusions of complete and His-lacking AA mixtures with or without CPA. Amino acids were purchased from US-Biochemical (Cleveland, OH), *para*-aminohippuric acid (PAH) was purchased from ICN Biochemicals (Aurora, OH), and CPA from Sigma Chemical Co. (Oakville, Ontario, Canada). Solutions of AA (147 g/L) and CPA (3 g/L saline) were prepared the day before infusion. All solutions were adjusted to pH 7.4 and filtered for sterility through a 0.22- $\mu$ m cellulose acetate filter into autoclaved polypropylene bottles. Amino acid solutions were infused for approximately 10 h between morning and evening milking through sterile Tygon surgical tubing into one iliac artery at  $5.0 \pm 0.2$  mL/min. Every 30 min, either saline or the CPA solution was infused at  $5.0 \pm 0.2$  mL/min for 5 min into the same artery as the AA. Infusion rates were measured by recording weights of infusate bottles several times during the day. The AA

infusion rate amounted to 44 g/h, and the infusion of CPA amounted to 150 mg/h.

Iliac plasma flow rate was estimated either from the arteriovenous concentration difference (AVD) of PAH included in the AA infusates at 16.6 g/L (Cant et al., 2002), or from the exponential decline of venous PAH concentration following a rapid injection of approximately 100 mg of PAH into the external iliac artery according to Qiao et al. (2005). In either case, at 8, 9, and 10 h into the infusion, blood samples were withdrawn with a syringe pump from the external iliac artery contralateral to infusion and the ipsilateral venous catheter simultaneously over a period of 10 min. We used second-lactation cows to avoid the reversal of blood flow in the pudendal veins that contaminates the mammary arteriovenous difference in cows of third lactation or higher (Thivierge et al., 2000). Venous anastomoses between right and left udder halves could also contaminate the unilateral arteriovenous difference but we have observed negligible crossover previously (Cant et al., 2001), and Metcalf et al. (1992) reported little or no crossover in cows when standing. Samples were collected from standing cows into EDTA Vacutainers (Becton Dickinson, Franklin Lakes, NJ) on ice. Plasma was separated by centrifugation for 20 min at  $1,500 \times g$  and  $4^\circ\text{C}$ , transferred to polypropylene tubes, and stored at  $-20^\circ\text{C}$  until analyzed. Milk samples were collected at the evening milking from each udder half and stored at  $5^\circ\text{C}$  for no more than 2 d before analysis.

Concentrations of PAH in plasma and infusates were measured as described previously (Cant et al., 2001). Arterial and venous samples from each cow were pooled daily and analyzed for glucose (Sigma kit no. 510-A; Raabo and Terkildsen, 1960), triacylglycerol (TAG; Sigma kit no. 336; McGowan et al., 1983), acetate (Boehringer Mannheim kit; R-Biopharm GmbH, Darmstadt, Germany; Bergmeyer and Moellering, 1983), BHBA (Cant et al., 1993), NEFA (NEFA C kit; Wako Chemicals GmbH, Neuss, Germany; Johnson and Peters, 1993), urea (Sigma kit no. 640-B; Chaney and Marbach, 1962), and  $\alpha$ -amino N (AAN; Evans et al., 1993). Long-chain fatty acid concentrations were calculated as  $3 \times \text{TAG} (\mu\text{M}) + \text{NEFA} (\mu\text{M})$ . Protein, fat, and lactose contents of milk samples were measured by infrared spectroscopy (AOAC, 1996).

Iliac plasma flow (L/h) was calculated as infusion rate of PAH (mg/h)/AVD of PAH (mg/L), or as dose of PAH (mg)/area under venous PAH curve (mg·h/L). Local mammary arterial concentrations of AAN were calculated as systemic arterial concentration + infused AAN/ilic plasma flow, and AVD of AAN was calculated as the difference between milk vein and local arterial concentrations. Mammary extraction percentages of blood metabolites were calculated as the ratio of AVD to arte-

rial concentration. A change in extraction can arise from a change in the rate constant for net uptake from the capillary,  $k$ , or the volume of perfused capillaries,  $\text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$ , due to local vasodilatory mechanisms. Thus, a  $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$  product was calculated as  $-\ln(1 - \text{extraction}) \times \text{ilic plasma flow}$  (Cant and McBride, 1995).

Variance in observations  $Y_{ijklm}$  was analyzed by the GLM procedure of SAS version 9 (SAS Institute Inc., Cary, NC) according to

$$Y_{ijklm} = \mu + \text{block}_i + \text{cow}(\text{block})_{j(i)} + \text{per}_k + \text{his}_l + \text{cpa}_m + \text{his} \times \text{cpa}_{lm} + \varepsilon_{ijklm}$$

where  $\mu$  = overall mean,  $\text{block}_i$  =  $i$ th effect of block ( $i = 1$  or  $2$ ),  $\text{cow}(\text{block})_{j(i)}$  =  $j$ th effect of cow within block ( $j = 1$  or  $2$ ),  $\text{per}_k$  =  $k$ th effect of period ( $k = 1$  to  $4$ ),  $\text{his}_l$  =  $l$ th effect of infused His level ( $l = 1$  or  $2$ ),  $\text{cpa}_m$  =  $m$ th effect of the histamine  $H_1$  receptor inhibitor ( $m = 1$  or  $2$ ),  $\text{his} \times \text{CPA}_{lm}$  = effect of interaction between the 2 main effects, and  $\varepsilon_{ijklm}$  = random variation, assumed to be  $N(0, \sigma^2)$ . Because of missing observations of metabolite concentrations on d 1 for one of the cows, results were expressed as least squares means. Statistical significance was declared at  $P < 0.05$  and trends were declared at  $0.05 < P \leq 0.15$ . To test for treatment carryover effects from one day to the next in the Latin square design, the previous day's treatment was included as a covariate in the ANOVA model. None of the previous day covariate effects were significant.

## RESULTS

The profile of the AA infusate and the calculated increase in AA concentrations of the blood supplying the infused half of the mammary gland are presented in Table 2. Based on measured rates of iliac plasma flow, the infusion increased the local mammary mean arterial plasma concentrations of essential AA and nonessential AA by 446 and 670  $\mu\text{M}$ , respectively.

Subtraction of His from the AA infusate had no effect on milk or component yields, fat or lactose percentages, but milk protein content tended to decrease from 3.98 to 3.79 ( $P = 0.07$ ) on average (Table 3). The histamine  $H_1$  receptor inhibitor CPA decreased feed intake by 20% ( $P = 0.02$ ) during the infusion and tended to decrease milk, protein, fat, and lactose yields ( $0.05 < P < 0.10$ ). There was no effect of CPA on the proportions of protein, fat, and lactose in milk. No interactions between His and CPA infusion were observed in the infused udder half.

Neither of the infusion treatments affected iliac plasma flow (Table 4). Systemic arterial concentrations of AAN tended to increase from an average of 3.18 to 3.42 mM ( $P = 0.09$ ) when His was removed from the



**Table 2.** Amino acid composition of infusate and estimated increase in mammary plasma AA concentrations at a plasma flow rate of 322 L/h

| AA                   | g/L   | Local increase in plasma concentration, $\mu M$ |
|----------------------|-------|---|
| Ala                  | 4.55  | 53  |
| Arg-HCl              | 4.84  | 24  |
| Asp                  | 10.56 | 82  |
| Cys                  | 1.32  | 8   |
| Glu                  | 28.60 | 202   |
| Gly                  | 2.64  | 37  |
| His-HCl <sup>1</sup> | 3.80  | 22  |
| Ile                  | 7.77  | 62  |
| Leu                  | 13.20 | 105   |
| Lys-HCl              | 11.59 | 57  |
| Met                  | 3.52  | 25  |
| Pro                  | 14.52 | 131   |
| Ser                  | 8.07  | 80  |
| Thr                  | 7.04  | 61  |
| Trp                  | 2.20  | 11  |
| Tyr                  | 13.49 | 77  |
| Val                  | 8.95  | 79  |
| Essential AA         | 63    | 446   |
| Nonessential AA      | 84    | 670   |
| HCl                  | 1.0   |   |
| NaOH                 | 10.0  |   |

<sup>1</sup>His was not present in all infusates.

infusate, but close arterial concentrations, extraction percentages, and uptakes were not affected. Histidine subtraction tended to increase mammary long-chain fatty acid uptake from 73 to 86 mmol/h ( $P = 0.15$ ). Infusion of CPA decreased arterial and venous concentrations of acetate by 25 ( $P = 0.02$ ) and 35% ( $P = 0.01$ ), respectively, and extraction increased from 76 to 80% ( $P = 0.01$ ). There was no effect of CPA on the  $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$  for acetate, indicating that the dynamics of acetate uptake were not affected by treatment. Net uptakes of acetate, in mmol/h, were 23% lower during CPA infu-

sion ( $P = 0.05$ ) because of the lower acetate concentrations in arterial plasma. The  $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$  for TAG and long-chain fatty acids tended to be decreased by CPA ( $0.05 < P < 0.10$ ).

## DISCUSSION

### Role of Histamine in Mammary Blood Flow

The hypothesis under test in this experiment consisted of 2 parts: first, that histamine release into the interstitial fluid surrounding the mammary vasculature is directly related to arterial His concentration, and second, that mammary blood flow is reduced by interstitial histamine via  $H_1$  receptors. According to this hypothesis, the effect of blocking the  $H_1$  receptor with CPA should be an increase in mammary blood flow. The effect of a reduced arterial His concentration should also be an increase in mammary blood flow. No effect of treatment on iliac plasma flow was observed, so the hypothesis is rejected.

A decrease in arterial His concentration when His was subtracted from the infusion of 44 g/h of AA was evidenced by the 0.22-percentage-unit decrease in milk protein content, which is similar in magnitude to the 0.16-unit decrease observed when His was subtracted from 30 g/h of the same infusate (Cant et al., 2001). No hyperemic response to the low His concentration was observed in the previous experiment (Cant et al., 2001) or in the current one. The results suggest that if histamine is indeed vasoactive in the mammary vasculature it is not released in proportion to concentration of its precursor, His.

Histamine is synthesized from His by histidine decarboxylase, and the rate of histamine synthesis is there-

**Table 3.** Least squares means of feed intake, milk yield, and milk contents from the infused udder half after 10 h of infusion of an AA solution with or without His, and saline or a solution of the histamine  $H_1$  receptor inhibitor chlorpheniramine (CPA), into one external iliac artery of 4 cows

| Item              | Treatment |      |        |      | SEM  | <i>P</i> -value <sup>1</sup> |      |           |
|-------------------|-----------|------|--------|------|------|------------------------------|------|-----------|
|                   | No His    |      | His    |      |      | His                          | CPA  | His × CPA |
|                   | No CPA    | CPA  | No CPA | CPA  |      |                              |      |           |
| Feed intake, kg/d | 17.7      | 14.1 | 18.2   | 14.5 | 1.1  | 0.71                         | 0.02 | 0.94      |
| Milk yield, g/h   | 545       | 427  | 539    | 400  | 57   | 0.78                         | 0.06 | 0.86      |
| Protein           |           |      |        |      |      |                              |      |           |
| %                 | 3.86      | 3.68 | 3.99   | 3.98 | 0.10 | 0.07                         | 0.35 | 0.39      |
| g/h               | 19.4      | 14.9 | 20.0   | 15.8 | 2.1  | 0.74                         | 0.09 | 0.95      |
| Fat               |           |      |        |      |      |                              |      |           |
| %                 | 4.41      | 4.32 | 4.50   | 4.45 | 0.27 | 0.68                         | 0.81 | 0.94      |
| g/h               | 22.3      | 18.6 | 23.2   | 17.7 | 2.0  | 1.00                         | 0.06 | 0.65      |
| Lactose           |           |      |        |      |      |                              |      |           |
| %                 | 4.32      | 4.49 | 4.36   | 4.41 | 0.10 | 0.81                         | 0.31 | 0.55      |
| g/h               | 23.4      | 19.2 | 23.4   | 17.6 | 2.4  | 0.76                         | 0.08 | 0.75      |
| Protein:fat       | 0.87      | 0.86 | 0.89   | 0.94 | 0.04 | 0.33                         | 0.73 | 0.49      |

<sup>1</sup>Probability of no effect of infusion of His, CPA, or their interaction.

**Table 4.** Least squares means of plasma flows and balance of plasma metabolites across the infused udder half of 4 cows during the last 3 h of a 10-h infusion of an AA solution with or without His, and saline or a solution of the histamine H<sub>1</sub> receptor inhibitor chlorpheniramine (CPA), into one external iliac artery

| Item  | Treatment |      |        |      | SEM  | <i>P</i> -value <sup>1</sup> |      |           |
|---|-----------|------|--------|------|------|------------------------------|------|-----------|
|   | No His    |      | His    |      |      | His                          | CPA  | His × CPA |
|   | No CPA    | CPA  | No CPA | CPA  |      |                              |      |           |
| Plasma flow, L/h  | 337       | 326  | 330    | 298  | 17   | 0.38                         | 0.29 | 0.60      |
| Glucose   |           |      |        |      |      |                              |      |           |
| Artery, mM  | 3.43      | 2.77 | 3.58   | 3.38 | 0.31 | 0.31                         | 0.25 | 0.51      |
| Vein, mM  | 2.66      | 2.08 | 2.58   | 2.54 | 0.18 | 0.39                         | 0.18 | 0.23      |
| Extraction, %   | 21.7      | 25.5 | 28.2   | 25.0 | 3.3  | 0.44                         | 0.93 | 0.37      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> <sup>2</sup> L/h | 94.1      | 94.1 | 109    | 87.6 | 11.7 | 0.76                         | 0.44 | 0.44      |
| Uptake, mmol/h  | 282       | 234  | 310    | 255  | 43   | 0.61                         | 0.31 | 0.94      |
| Acetate   |           |      |        |      |      |                              |      |           |
| Artery, mM  | 1.10      | 0.85 | 1.19   | 0.86 | 0.08 | 0.58                         | 0.02 | 0.68      |
| Vein, mM  | 0.25      | 0.18 | 0.30   | 0.18 | 0.02 | 0.40                         | 0.01 | 0.23      |
| Extraction, %   | 77.3      | 79.1 | 74.8   | 81.0 | 0.9  | 0.79                         | 0.01 | 0.08      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> , L/h            | 496       | 497  | 455    | 492  | 34   | 0.55                         | 0.63 | 0.65      |
| Uptake, mmol/h  | 288       | 229  | 285    | 210  | 24   | 0.68                         | 0.05 | 0.76      |
| BHBA  |           |      |        |      |      |                              |      |           |
| Artery, mM  | 1.35      | 1.42 | 1.39   | 1.28 | 0.19 | 0.86                         | 0.83 | 0.91      |
| Vein, mM  | 0.75      | 0.89 | 0.81   | 0.66 | 0.10 | 0.46                         | 0.99 | 0.23      |
| Extraction, %   | 42.4      | 38.5 | 40.7   | 47.5 | 4.0  | 0.44                         | 0.74 | 0.27      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> , L/h            | 190       | 154  | 174    | 194  | 21   | 0.62                         | 0.74 | 0.29      |
| Uptake, mmol/h  | 202       | 176  | 195    | 197  | 43   | 0.89                         | 0.80 | 0.78      |
| Triacylglycerol   |           |      |        |      |      |                              |      |           |
| Artery, μM  | 140       | 142  | 137    | 137  | 13   | 0.81                         | 0.94 | 0.96      |
| Vein, μM  | 66        | 77   | 69     | 72   | 7    | 0.92                         | 0.44 | 0.64      |
| Extraction, %   | 53.0      | 45.0 | 51.3   | 49.3 | 3.6  | 0.75                         | 0.26 | 0.47      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> , L/h            | 276       | 199  | 250    | 210  | 23   | 0.78                         | 0.06 | 0.50      |
| Uptake, mmol/h  | 26.0      | 21.9 | 22.7   | 19.7 | 3.0  | 0.42                         | 0.32 | 0.87      |
| NEFA  |           |      |        |      |      |                              |      |           |
| Artery, μM  | 135       | 144  | 117    | 137  | 18   | 0.57                         | 0.50 | 0.80      |
| Vein, μM  | 94        | 101  | 93     | 99   | 11   | 0.95                         | 0.60 | 0.96      |
| Long-chain fatty acids                                  |           |      |        |      |      |                              |      |           |
| Artery, μM  | 554       | 568  | 529    | 549  | 35   | 0.58                         | 0.66 | 0.94      |
| Vein, μM  | 292       | 331  | 301    | 315  | 25   | 0.90                         | 0.37 | 0.67      |
| Extraction, %   | 48.4      | 42.3 | 45.5   | 45.7 | 3.1  | 0.95                         | 0.42 | 0.39      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> , L/h            | 238       | 184  | 214    | 191  | 17   | 0.65                         | 0.09 | 0.45      |
| Uptake, mmol/h  | 92.3      | 79.7 | 76.5   | 69.8 | 7.0  | 0.15                         | 0.26 | 0.71      |
| α-Amino N   |           |      |        |      |      |                              |      |           |
| Opposite artery, mM                                     | 3.54      | 3.30 | 3.14   | 3.21 | 0.11 | 0.09                         | 0.49 | 0.25      |
| Artery, mM  | 4.66      | 4.34 | 4.25   | 4.36 | 0.11 | 0.17                         | 0.43 | 0.14      |
| Vein, mM  | 3.87      | 3.87 | 3.54   | 3.66 | 0.22 | 0.30                         | 0.83 | 0.81      |
| Extraction, %   | 16.7      | 10.6 | 16.4   | 16.4 | 4.1  | 0.56                         | 0.52 | 0.53      |
| k·Vol <sub>cap</sub> ·N <sub>cap</sub> , L/h            | 66.8      | 40.0 | 61.0   | 54.9 | 16.3 | 0.81                         | 0.39 | 0.58      |
| Uptake, mmol/h  | 283       | 167  | 229    | 204  | 58   | 0.91                         | 0.32 | 0.50      |
| Urea  |           |      |        |      |      |                              |      |           |
| Artery, mM  | 3.52      | 3.59 | 3.12   | 3.45 | 0.40 | 0.56                         | 0.67 | 0.77      |

<sup>1</sup>Probabilities of no effect of infusion of His, CPA, or their interaction.<sup>2</sup>k·Vol<sub>cap</sub>·N<sub>cap</sub> = -Ln (1 - extraction) × iliac plasma flow (Cant and McBride, 1995).

fore expected to follow a dependence on substrate concentration, but histamine can be stored intracellularly before release. Most of the histamine stored in the body is in mast cells and basophils of the immune system. The majority of histamine in the mammary glands is also located in mast cells but histamine and histidine decarboxylase are also present in the mammary epithelium (Maslinski et al., 1993; Wagner et al., 2003). Upon stimulation of release, dependent on the cell type and anatomical location, histamine acts in various roles as a neurotransmitter or a signal of inflammation and

other immune responses, gastric acid secretion, or smooth muscle contraction. After a week of dosing rats orally with 1 g/kg of His twice daily, histamine concentrations in regions of the brain were significantly elevated, but concentrations of the metabolite *tele*-methyl-histamine, indicating histamine release, were not affected (Lozeva et al., 2003). Thus, it appears that release of histamine is a stronger controller of interstitial histamine concentration than concentration of His. Plasma histamine concentrations were also not affected by the His dosing (Lozeva et al., 2003). These observa-

tions support rejection of the hypothesis that a reduced arterial His concentration affects histamine concentration in the mammary vasculature of the lactating dairy cow.

The lack of a blood flow response to the H<sub>1</sub> receptor blocker CPA allows us to reject the second part of the hypothesis that histamine is vasoactive in the mammary vasculature via H<sub>1</sub> receptors. Histamine has been shown to exert a combination of constrictive and dilatory effects on various arteries, each of which are mediated through separate response mechanisms. There are 4 histamine receptors, designated H<sub>1</sub> to H<sub>4</sub> (Wagner et al., 2003). Antagonists of either the H<sub>1</sub> or H<sub>2</sub> receptor, but not both in the same artery, have abolished the vasodilatory effect of histamine (Jin et al., 2006; Stähli et al., 2006), which is dependent on precontraction of the artery and the presence of an intact endothelium (Jin et al., 2006; Stähli et al., 2006). The net effect of histamine on isolated arteries at greater concentrations is constrictive. The vasoconstriction occurs in the absence of endothelial cells but is abolished by analogs of the H<sub>1</sub> receptor (Jin et al., 2006; Stähli et al., 2006). The vasoconstriction is thus considered to be due to a direct effect of histamine on the arterial smooth muscle or vascular nerves. Internal mammary arteries of the human were found to be dilatory to histamine at low concentrations, whereas radial arteries were not (Stähli et al., 2006). Both constricted at higher histamine concentrations. The pudendal arteries of nonlactating goats only responded to histamine with vasoconstriction (Jakobsen et al., 1994). In addition, constriction of the caprine pudendal arteries was inhibited by an H<sub>1</sub> receptor blocker (Jakobsen et al., 1994). If the histamine response network behaves in the vasoactive milieu of the mammary glands in the same way it did in isolated arteries *in vitro*, then the antagonist of the H<sub>1</sub> receptor should have relaxed resistance to flow, just as blockers of the adenosine and NO responses affect mammary plasma flow (Lacasse et al., 1996; Madsen et al., 2001). However, we observed no effect of CPA on mammary plasma flow.

An issue that needs to be addressed in interpretation of our plasma flow results is that the measured plasma flow was not directed exclusively to the mammary glands but included femoral arterial flow to the hind limb. An effect of CPA on plasma flow to the hind limb could, therefore, either exaggerate or mask effects on mammary plasma flow. As with other arteries, both dilation and constriction of the cutaneous and femoral arteries have been observed, mediated by H<sub>1</sub> receptors (Clough et al., 1998; Arai and Chiba, 1999), so there is no consensus on how blood flow to the hind limb might be affected by CPA infusion. However, as discussed by Cant et al. (2002) and in agreement with measurement

in lactating goats of blood flow simultaneously in the external iliac and external pudendal arteries (Bequette et al., 2001), mammary blood flow accounts for at least 80% of the iliac flow. This means that even a relatively large change in flow to the hind limb will have only minor effects on the iliac flow. Thus, our conclusion is that H<sub>1</sub> receptors in the mammary gland are not involved in bovine mammary blood flow regulation *in vivo*.

### ***Role of Histamine in Milk Secretion***

Although H<sub>1</sub> receptors did not influence mammary blood flow, there was a local effect of histamine mediated by H<sub>1</sub> receptors on milk yield. Yield from the infused udder half was negatively affected by infusion of CPA, whereas no effect was seen on milk yield from the noninfused udder half (data not shown). It has been suggested that histamine plays a role in milk ejection through effects on smooth muscle contraction (Eriksson et al., 1999). If this effect is mediated by H<sub>1</sub> receptors, it would explain the negative effect of CPA on milk yield. Milk fat content decreased by 16% when milk ejection was inhibited by continuous epinephrine infusion into goats (Leenanuruxsa and McDowell, 1985) but we observed no effect on percentages of protein, fat, or lactose in milk.

The observation that feed intake dropped when CPA was infused indicates that CPA not only acted locally but also had a systemic effect. Intraperitoneal injection of the H<sub>1</sub> blocker dexbrompheniramine into goats at 2 mg/kg of BW<sup>0.75</sup> did not affect feed intake (Rossi et al., 1998). Our arterial infusion over 10 h amounted to a 6-fold higher dose of 12 mg/kg of BW<sup>0.75</sup> chlorpheniramine. Histamine in several species affects gastrointestinal blood flow by a vasodilatory effect mediated by H<sub>1</sub> receptors (Buckley et al., 1988; Rydning et al., 2001). It is therefore possible that blockade of the H<sub>1</sub> receptors over 10 h compromised gastrointestinal blood flow and thereby affected digestion and feed intake negatively. A direct effect on appetite centers in the brain does not seem likely, because histamine actually depresses feed intake and blockade of the H<sub>1</sub> receptors attenuates this effect (Masaki et al., 2001).

The decrease in arterial concentration of acetate when CPA was infused was likely a consequence of the putative CPA effect on the gastrointestinal tract because endogenous production of acetate is relatively low compared with the exogenous supply in ruminants (Pethick et al., 1981). The 25% lower acetate concentration resulted in a 23% decrease in acetate uptake by the mammary glands, but no change in milk output of its product, fat, relative to the other products, protein and lactose.

There was also a decrease ( $P = 0.06$ ) in the  $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$  product for TAG with CPA infusion that accounted for a decrease ( $P = 0.09$ ) in  $k \cdot \text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$  for long-chain fatty acids. Because iliac plasma flow was not affected, the volume of perfused capillaries,  $\text{Vol}_{\text{cap}} \cdot N_{\text{cap}}$ , can be assumed to have remained constant between treatments, meaning that a drop in efficiency,  $k$ , of TAG uptake occurred. Disappearance of TAG between artery and vein is due to activity of endothelial lipoprotein lipase. We are unaware of a connection between the  $H_1$  receptor and lipoprotein lipase activity, but there is a connection between histamine and heparin that may be significant. Heparin is stored in and released from mast cells, which also happen to be the major site of histamine storage in the body and the mammary glands (Maslinski et al., 1993). Heparin in the vascular lumen knocks mature lipoprotein lipase off the endothelium to severely decrease arteriovenous TAG hydrolysis (Augustus et al., 2003). Antagonism of the  $H_1$  receptor may have interfered with the control of heparin release from mast cells and caused disengagement of lipoprotein lipase from the mammary endothelium. Although efficiency of TAG hydrolysis may have been reduced by CPA, net uptake of long-chain fatty acids across the mammary glands was not significantly affected.

Finding that CPA decreased feed intake and circulating concentrations of a milk precursor (acetate) raises the possibility that the reduced nutrient supply may have elicited a decrease in the rate of synthesis of milk components. A lower milk synthesis rate would be expected to decrease mammary blood flow (Linzell, 1974; Nielsen et al., 1990), countervailing the increase in mammary blood flow expected from the working hypothesis behind this experiment. However, the decline in milk yield in cows severely restricted in DMI did not commence until after 18 h of restriction (Toerien and Cant, 2007), and complete starvation of goats only caused a 10% decrease in milk yield after 8 h (Linzell, 1967). Thus, the likelihood of a nutrition-mediated decrease in mammary blood flow in this experiment, masking a CPA-mediated increase in flow, is negligible.

### Role of AA in Mammary Blood Flow

It has been suggested that a deficient exogenous supply of individual AA may influence the rate of mammary blood flow in ruminants (Guinard and Rulquin, 1995). One of the AA that has been identified as potentially deficient for milk production is His (Vanhatalo et al., 1999). In the present study, cows were fed a low-protein diet and supplied with 44 g/h of exogenous AA, equivalent to approximately one-third of the total metabolizable AA supply, with or without His. Although a temporary His deficiency was apparent in the milk protein

depression typical of the short-term infusion protocol, and in the increase in plasma AAN concentration, iliac plasma flow did not change. This finding lies in contrast to the 33% increase in mammary blood flow observed in goats subjected to a deficiency of approximately 45% of the metabolizable His supply for 6 d (Bequette et al., 2000). The hyperemia may require a few days to develop because of responses to the His deficiency that occur in extramammary organs of the cow. Although we have previously shown, with a simulation model of ATP balance in the milk-synthesizing system (Cant et al., 2003), that changes in circulating concentrations of energy metabolites due to extramammary effects can account for mammary hyperemias observed in response to both Met (Guinard and Rulquin, 1995) and His (Bequette et al., 2000) supply, no rigorous test of that hypothesis has been conducted. The mechanism by which an amino acid deficiency influences mammary blood flow does not involve histamine signaling through the  $H_1$  receptor and remains unidentified.

### ACKNOWLEDGMENTS

A special thanks to Sarah Armstrong, Jodi Calberry, Sue Edmonds, Suzy Kilby, and John Sleeman for helping with sampling and taking care of the animals. Financial support for this work was provided by NSERC Canada, Agribands Purina Canada, and the Ontario Ministry of Agriculture and Food. Norm Purdie was in receipt of a Dairy Research Development Corporation postgraduate award. Torben Gosvig Madsen was in receipt of a postgraduate award from the Faculty of Life Sciences, Copenhagen University (formerly Royal Veterinary and Agricultural University), Denmark.

### REFERENCES

- AOAC. 1996. Official Methods of Analysis. 16th ed. AOAC, Arlington, VA.
- Arai, M., and S. Chiba. 1999. Endothelium-dependent vasodilation mechanisms by histamine in simian but not in canine femoral arterial branches. *J. Auton. Pharmacol.* 19:267–273.
- Augustus, A. S., Y. Kako, H. Yagyu, and I. J. Goldberg. 2003. Routes of FA delivery to cardiac muscle: Modulation of lipoprotein lipolysis alters uptake of TG-derived FA. *Am. J. Physiol.* 284:E331–E339.
- Bergmeyer, H. U., and H. Moellering. 1983. Acetate: Determination with acetate kinase. Pages 628–639 in *Methods of Enzymatic Analysis*. 3rd ed. Vol. 6. H. U. Bergmeyer, ed. Acad. Press, New York, NY.
- Bequette, B. J., M. D. Hanigan, A. G. Calder, C. K. Reynolds, G. E. Lobley, and J. C. MacRae. 2000. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. *J. Dairy Sci.* 83:765–775.
- Bequette, B. J., C. E. Kyle, L. A. Crompton, V. Buchan, and M. D. Hanigan. 2001. Insulin regulates milk production and mammary gland and hind-leg amino acid fluxes and blood flow in lactating goats. *J. Dairy Sci.* 84:241–255.
- Buckley, N. M., S. Diamant, I. D. Frasier, and K. Owusu. 1988. Histamine or adenosine blockade alters intestinal blood flow autoregulation in swine. *Am. J. Physiol.* 254:G156–G161.



- Cant, J. P., R. Berthiaume, H. Lapierre, P. H. Luimes, B. W. McBride, and D. Pacheco. 2003. Responses of the bovine mammary glands to absorptive supply of single amino acids. *Can. J. Anim. Sci.* 83:341–355.
- Cant, J. P., E. J. DePeters, and R. L. Baldwin. 1993. Mammary uptake of energy metabolites in dairy cows fed fat and its relationship to milk protein depression. *J. Dairy Sci.* 76:2254–2265.
- Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. *J. Dairy Res.* 62:405–422.
- Cant, J. P., D. R. Trout, F. Qiao, and B. W. McBride. 2001. Milk composition responses to unilateral arterial infusion of complete and histidine-lacking amino acid mixtures to the mammary glands of cows. *J. Dairy Sci.* 84:1192–1200.
- Cant, J. P., D. R. Trout, F. Qiao, and N. G. Purdie. 2002. Milk synthetic response of the bovine mammary gland to an increase in the local concentration of arterial glucose. *J. Dairy Sci.* 85:494–503.
- Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8:130–132.
- Clough, G. F., A. R. Bennett, and M. K. Church. 1998. Effects of H1 antagonists on the cutaneous vascular response to histamine and bradykinin: A study using laser Doppler imaging. *Br. J. Dermatol.* 138:806–814.
- Davis, S. R., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. *J. Dairy Sci.* 68:1041–1058.
- Eriksson, L., W. A. Fogel, S. Eklund-Uusitalo, L. Tuomisto, and C. Maslinski. 1999. Is histamine involved in milk ejection in goats? *Inflamm. Res.* 48:S90–S91.
- Evans, P. C., F. M. Ffolliott-Powell, and J. E. Harding. 1993. A colorimetric assay for amino nitrogen in small volumes of blood: Reaction with  $\beta$ -naphthoquinone sulfonate. *Anal. Biochem.* 208:334–337.
- Guinard, J., and H. Rulquin. 1995. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. *J. Dairy Sci.* 78:2196–2207.
- Jakobsen, K., E. O. Mikkelsen, and M. O. Nielsen. 1994. Studies on responses to potassium, noradrenaline, serotonin, histamine and prostaglandin F2alpha, of isolated pudendal arteries from non-lactating goats. *Comp. Biochem. Physiol.* 109C:167–172.
- Jin, H., T. Koyama, Y. Hatanaka, S. Akiyama, F. Takayama, and H. Kawasaki. 2006. Histamine-induced vasodilation and vasoconstriction in the mesenteric resistance artery of the rat. *Eur. J. Pharmacol.* 529:136–144.
- Johnson, M. J., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. *J. Anim. Sci.* 71:753–756.
- Lacasse, P., V. C. Farr, S. R. Davis, and C. G. Prosser. 1996. Local secretion of nitric oxide and the control of mammary blood flow. *J. Dairy Sci.* 79:1369–1374.
- Leenanuruksa, D., and G. H. McDowell. 1985. Effects of prolonged intravenous infusions of adrenaline on glucose utilization, plasma metabolites, hormones and milk production in lactating sheep. *Aust. J. Biol. Sci.* 38:197–208.
- Linzell, J. L. 1967. The effect of very frequent milking and of oxytocin on the yield and composition of milk in fed and fasted goats. *J. Physiol.* 190:333–346.
- Linzell, J. L. 1974. Mammary blood flow and methods of identifying and measuring precursors of milk. Pages 143–225 in *Lactation, A Comprehensive Treatise*. Vol. 1. B. L. Larson and V. R. Smith, ed. Academic Press, New York, NY.
- Lozeva, V., J. Tarhanen, M. Attila, P. T. Männistö, and L. Tuomisto. 2003. Brain histamine and histamine H3 receptors following repeated L-histidine administration in rats. *Life Sci.* 73:1491–1503.
- Madsen, T. G., D. R. Trout, S. Cieslar, N. G. Purdie, M. O. Nielsen, and J. P. Cant. 2001. Effects of N-nitro-arginine on blood flow and nutrient uptake in the mammary glands of dairy cows. *J. Dairy Sci.* 84(Suppl. 1):390. (Abstr.)
- Masaki, T., H. Yoshimatsu, S. Chiba, K. Watanabe, and T. Sakata. 2001. Targeted disruption of histamine H<sub>1</sub> receptor attenuates regulatory effects of leptin on feeding, adiposity, and UCP family in mice. *Diabetes* 50:385–391.
- Maslinski, C., D. Kierska, W. A. Fogel, A. Kinnunen, and P. Panula. 1993. Histamine: Its metabolism and localization in mammary gland. *Comp. Biochem. Physiol.* 105C:269–273.
- McGowan, M. W., J. D. Artiss, D. R. Strandbergh, and B. Zak. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* 29:538–542.
- Metcalf, J. A., S. J. Roberts, and J. D. Sutton. 1992. Variations in blood flow to and from the bovine mammary gland measured using transit time ultrasound and dye dilution. *Res. Vet. Sci.* 53:59–63.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Nielsen, M. O., I. R. Fleet, K. Jakobsen, and R. B. Heap. 1995. The local differential effect of prostacyclin, prostaglandin E2 and prostaglandin F2 alpha on mammary blood flow of lactating goats. *J. Endocrinol.* 145:585–591.
- Nielsen, M. O., K. Jakobsen, and J. N. Jorgensen. 1990. Changes in mammary blood flow during the lactation period in goats measured by the ultrasound Doppler principle. *Comp. Biochem. Physiol.* 97A:519–524.
- Parsons, G. H., A. C. Villablanca, J. M. Brock, R. S. Howard, S. R. Colbert, G. M. Nichol, and K. F. Chung. 1992. Bronchial vasodilation by histamine in sheep: Characterization of receptor subtype. *J. Appl. Physiol.* 72:2090–2098.
- Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. J. Northrop. 1981. Acetate supply and utilization by the tissue of sheep in vivo. *Br. J. Nutr.* 46:97–110.
- Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse. 1996. Regulation of blood flow in the mammary microvasculature. *J. Dairy Sci.* 79:1184–1197.
- Qiao, F., D. R. Trout, V. M. Quinton, and J. P. Cant. 2005. A compartmental capillary, convolution integration model to investigate nutrient transport and metabolism in vivo from paired indicator/nutrient dilution curves. *J. Appl. Physiol.* 99:788–798.
- Raabo, E., and T. C. Terkildsen. 1960. On the enzymatic determination of blood glucose. *Scand. J. Clin. Lab. Invest.* 12:402–407.
- Rossi, R., E. Del Prete, and E. Scharer. 1998. Effects of histamine H<sub>1</sub> receptors on the feeding and drinking patterns in pygmy goats. *J. Dairy Sci.* 81:2369–2375.
- Rydning, A., O. Lyng, B. L. Adamsen, S. Falkmer, A. K. Sandvik, and J. E. Grønbech. 2001. Mast cells are involved in the gastric hyperemic response to acid back diffusion via release of histamine. *Am. J. Physiol.* 280:G1061–G1069.
- Satoh, H., and J. Inui. 1984. Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. *Eur. J. Pharmacol.* 97:321–324.
- Stähli, B. E., H. Greutert, S. Mei, P. Graf, K. Frischknecht, M. Stalder, L. Englberger, A. Künzli, L. Schärer, T. F. Lüscher, T. P. Carrel, and F. C. Tanner. 2006. Absence of histamine-induced nitric oxide release in the human radial artery: Implications for vasospasm of coronary artery bypass vessels. *Am. J. Physiol.* 290:H1182–H1189.
- Thivierge, M. C., D. Petitclerc, J. F. Bernier, Y. Couture, and H. Lapierre. 2000. External pudic venous reflux into the mammary vein in lactating dairy cows. *J. Dairy Sci.* 83:2230–2238.
- Toerien, C. A., and J. P. Cant. 2007. Duration of a severe feed restriction required to reversibly decrease milk production in the high-producing dairy cow. *Can. J. Anim. Sci.* 87:455–458.
- Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. *J. Dairy Sci.* 82:2674–2685.
- Wagner, W., A. Ichikawa, S. Tanaka, P. Panula, and W. A. Fogel. 2003. Mouse mammary epithelial histamine system. *J. Physiol. Pharmacol.* 54:211–223.
- Weekes, T. L., P. H. Luimes, and J. P. Cant. 2006. Responses to amino acid imbalances and deficiencies in lactating dairy cows. *J. Dairy Sci.* 89:2177–2187.